PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference D3-A0304P	**FOR FURTHER ACTION**	See Form PCT/IPEA/416
International application No. PCT/JP2004/009370	International filing date *(day/month/year)* 25.06.2004	Priority date *(day/month/year)* 27.06.2003
International Patent Classification (IPC) or national classification and IPC C07K14/505, A61K48/00, C12N5/06, C12N15/62		
Applicant DNAVEC RESEARCH INC. et al.		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 9 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *sent to the applicant and to the International Bureau)* a total of 13 sheets, as follows:
      ☒ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:
   - ☒ Box No. I Basis of the opinion
   - ☒ Box No. II Priority
   - ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - ☐ Box No. IV Lack of unity of invention
   - ☒ Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - ☒ Box No. VI Certain documents cited
   - ☐ Box No. VII Certain defects in the international application
   - ☐ Box No. VIII Certain observations on the international application

Date of submission of the demand	Date of completion of this report
21.01.2005	04.04.2005
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Pilat, D Telephone No. +49 89 2399-8668

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/JP2004/009370

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3 and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4)
   - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

   Description, Pages

   1-50 as originally filed

   Sequence listings part of the description, Pages

   1-13 received on 21.01.2005 with letter of 14.01.2005

   Claims, Numbers

   1-15 as originally filed

   Drawings, Sheets

   1/13-13/13 as originally filed

   ☒ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☐ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/JP2004/009370

Box No. II Priority

1. ☐ This report has been established as if no priority had been claimed due to the failure to furnish within the prescribed time limit the requested:
   - ☐ copy of the earlier application whose priority has been claimed (Rule 66.7(a)).
   - ☐ translation of the earlier application whose priority has been claimed (Rule 66.7(b)).

2. ☐ This report has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rule 64.1). Thus for the purposes of this report, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:

   see separate sheet

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application,

   ☒ claims Nos. 1-11

   because:

   ☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (specify):

   ☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☒ no international search report has been established for the said claims Nos. 1-11

   ☐ the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:

the written form	☐	has not been furnished
	☐	does not comply with the standard
the computer readable form	☐	has not been furnished
	☐	does not comply with the standard

   ☐ the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

   ☐ See separate sheet for further details

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/JP2004/009370

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	
	No:	Claims	1-6,8-15
Inventive step (IS)	Yes:	Claims	7
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	12-15
	No:	Claims	

2. Citations and explanations (Rule 70.7):

see separate sheet

Box No. VI Certain documents cited

1. Certain published documents (Rule 70.10)

and / or

2. Non-written disclosures (Rule 70.9)

see separate sheet

Supplemental Box relating to Sequence Listing

Continuation of Box I, item 2:

1. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this report has been established on the basis of:

   a. type of material:

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☒ in written format

   ☒ in computer readable form

   c. time of filing/furnishing:

   ☐ contained in the international application as filed

   ☐ filed together with the international application in computer readable form

   ☒ furnished subsequently to this Authority for the purposes of search and/or examination

   ☒ received by this Authority as an amendment on21.1.2005

2. ☒ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional observations, if necessary:

Ad Section I: Basis of the report

1. Reference is made to the following documents:

D1: KUME AKIHIRO ET AL: "In vivo expansion of transduced murine hematopoietic cells with a selective amplifier gene." THE JOURNAL OF GENE MEDICINE. MAR 2003, vol. 5, no. 3, March 2003 (2003-03), pages 175-181, XP009039186 ISSN: 1099-498X

D2: HANAZONO Y ET AL: "In vivo selective expansion of gene-modified hematopoietic cells in a nonhuman primate model" GENE THERAPY, vol. 9, no. 16, August 2002 (2002-08), pages 1055-1064, XP002303770 ISSN: 0969-7128

D3: NAGASHIMA TAKEYUKI ET AL: "New selective amplifier genes containing c-Mpl for hematopoietic cell expansion." BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, vol. 303, no. 1, 28 March 2003 (2003-03-28), pages 170-176, XP002303771 ISSN: 0006-291X

D4: JIN LIQING ET AL: "In vivo selection using a cell-growth switch" NATURE GENETICS, vol. 26, no. 1, September 2000 (2000-09), pages 64-66, XP002303772 ISSN: 1061-4036

D5: KROSL JANA ET AL: "Interleukin-3 (IL-3) inhibits erythropoietin-induced differentiation in Ba/F3 cells via the IL-3 receptor alpha subunit" JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 271, no. 44, 1996, pages 27432-27437, XP002303773 ISSN: 0021-9258

D6: SHIKAMA YAYOI ET AL: "A constitutively activated chimeric cytokine receptor confers factor-independent growth in hematopoietic cell lines" BLOOD, vol. 88, no. 2, 1996, pages 455-464, XP002303774 ISSN: 0006-4971

Ad Section II :Priority

2) The priority document pertaining to the present application was available at the time of establishing this IPER. It is seems that all claims enjoy priority rights from the filing date of the priority document. The documents indicated in the search report as P-documents are not to be regarded as state of the art according to Article 33 (2) PCT, as the date of priority claimed can be allowed for claims 1 to 15 of the present application, cf. Articles 33 (2) and 8 PCT.

Ad Section III :Non-establishment of opinion

3. Claims 1-11 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(i) PCT).

Ad Section V :Reasoned statement under Rule 66.2(a)(ii); citations and explanations supporting such statement

4. Novelty (Article 33 (2) PCT)

4.1 D1 Kume et al. describes 'selective amplifier genes' (SAGs) that encode chimeric proteins that are a fusion of granulocyte colony-stimulating factor receptor and the steroid-binding domain. Prototype SAGs conferred estrogen-responsive growth on murine hematopoietic progenitors. A detailed study of lineage showed a preferential expansion of EGFP(+) cells in granulocytes and monocytes following 4-hydroxytamoxifen administration. A granulocyte colony-stimulating factor receptor was linked to the estrogen receptor (see abstract). Bone marrow cells were transduced with the retroviral construct (see p.177 col.1 third paragraph). Subsequently SAG-transduced cells were tracked in a murine bone marrow transplantation model. Analysis of the impact of 4-hydroxytamoxifen stimulation was investigated (see p.178 col.1 2 full paragraph).

4.2 D2 Hanazono et al. describes a selective amplifier gene (SAG) consisting of a chimeric gene composed of the granulocyte colony-stimulating factor (G-CSF) receptor gene and the oestrogen receptor gene hormone-binding domain (see Fig.1). In the present study, the efficacy of the SAG in the setting of a clinically applicable cynomolgus monkey transplantation protocol was evaluated. Cynomolgus bone marrow CD34+ cells were transduced with retroviral vectors encoding the SAG and reinfused into each myeloablated monkey. Even with nonmyeloablative conditioning, successful engraftment of transduced cells even at low levels may allow expansion to clinically relevant levels with this method (see p.1059 col.1 1 full §). A modified SAG

with thrombopoietin receptor (Mpl) as a growth signal generator instead of G-CSF receptor to overcome variable responses among monkeys is proposed (see p.1060 col.2 last sentence of the 1 full paragraph).

4.3 D3 Nagashima et al. describes the in vitro cell expansion with modified SAGs containing the thrombopoietin (TPO) receptor (c-Mpl) gene instead of GCR as a more potent signal generator.

4.4 D4 Jin et al. describes the successful in vivo expansion of gene modified haemátopoietic cells using the cell growth switch composed of the intracellular part of Mpl and FKBP in a murine model. FKBP is a cytokine receptor-FK506 binding protein.

Thus, in view of the content of D1, D2, D3, D4 claims 1-6,8-13 lack novelty.

4.5 D5 Krosl et al. discloses that a chimeric receptor of the extracellular domain of the EpoR and the transmembrane and intracellular domains of IL-3R-beta-$_{IL-3}$ chain (EpoR/IL-3R-beta-$_{IL-3}$) was capable of Epo-induced proliferative and differentiating signalling. An EpoR/IL-3R-alpha chimera, in contrast, was capable of transmitting a weak Epo-induced proliferative signal but failed to stimulate accumulation of beta-globin mRNA (see abstract). EpoR chimeric cDNAs were generated (see materials and methods).
D6 Shikama et al. constructed four hybrid receptors: the extracellular region of either murine nEpoR or cEpoR linked to the transmembrane and cytoplasmic regions of either the human GMR-alpha or beta-c subunit (nE-alpha, nE-beta, cE-alpha, and cE-beta). Expression nEpo-beta led to Epo-dependent growth (see abstract). Hybrid and full length receptor were constructed and transfected into BaF3 or CTLL-2 cell lines (see materials and methods).

In view of the content of D5 and D6, claims 14 and 15 lack novelty.

4.6 None of the document cited in the international search report seems to disclose a method as claimed in claim 7. Thus, claim 7 seems novel.

5 Inventive step (Article 33 (3) PCT)

None of the document cited in the international search report, taken alone or in any combination, seems to suggest a method as claimed in claim 7. Accordingly, claim 7 seems to involve an inventive step.